ESTIMATION OF TOTAL PHENOLIC CONTENT OF *Emblica officinalis* AND *Curcuma domestica* COLD INFUSION (Haridramalake sheetha kashaya) AND ITS EFFECT ON THE FASTING BLOOD SUGAR OF PATIENTS WITH MILD DIABETES MELLITUS

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Submitted in partial fulfillment of the requirement for the award of the degree of

MASTER OF SCIENCE IN INDUSTRIAL UTILIZATION OF MEDICINAL AND AROMATIC PLANTS

of the

UNIVERSITY OF SRI JAWA WARDENEPURA

SRI LANKA

2010
DECLARATION

I do hereby declare that the work reported in this project report/thesis was exclusively carried out by me under the supervision of Prof. Ajith Abeysekera. It describes the result of my own independent research except where due reference has been made in the text. No part of this project report/thesis has been submitted earlier or concurrently for the same or any other degree.

Date: 2010/09/02

Signature of the Candidate

Certified by:

1. Supervisor (Name):................................. Date:.........................
   (Signature):........................................

2. Co-Supervisor (Name):............................ Date:.........................
   (Signature):.......................................
Ayurvedic herbal medicines have been used since ancient times to treat diabetes mellitus and many control trials have been performed to investigate their efficacy. The current review focuses on a herbal drug preparation which is used as a treatment for acute stage of diabetes mellitus. According to ayurvedic texts, Haridramalakee cold infusion has been used for diabetic mellitus since ancient time. This cold infusion contains Turmeric powder (Curcuma domestica) and fresh Nelli juice (Emblica officinalis).

This study was performed to determine the total phenolic content of Haridramalakee cold infusion every 2 hours from the initial stage up to 12 hours by UV-visible Spectrophotometer by using Folin-Denis reagent and gallic acid as the standard.

A clinical study was designed to investigate the hypoglycemic effect of Haridramalakee cold infusion in type 2 diabetes mellitus at the Ayurvedic teaching hospital, Borella. 10 patients having type 2 diabetes mellitus was given this cold infusion for 6 days without other medications, and the fasting blood glucose level was investigated on the 7th day.

According to the findings of the research, the total Poly-phenolic content of the Haridramalakee cold infusion increased with time, but rate of increase is less compared to the total poly-phenolic content of pure fresh Emblica officinalis juice. This gives an idea about the effect of turmeric powder on the rate of increase in total poly-phenols of Haridramalakee cold infusion. Water soluble poly-phenol content in Curcuma domestica powder was found to be very low. Intensity of yellow colour due to curcumin pigments increased with time in the Haridramalakee cold infusion. This shows that amount of
curcumin dissolved increases with time. Therefore it is evident that the anti-diabetic effect of the drug increases with time.

From the clinical study it was revealed that Haridramalakee cold infusion has significant hypoglycemic activity. Blood glucose level was decreased in all patients, and the blood glucose content was brought into normal range (110-120 mg/dl) in 60% of patients who had fasting blood glucose level below 200 mg/dl before treatment.
ACKNOWLEDGEMENT

It is with great pleasure to place on record my deepest gratitude to my supervisor Prof. A.M.Abeysekera, Dean, faculty of Applied science, university of Sri Jayewardenepura, Nugegoda, for his invaluable advice and guidance provided throughout the study and for giving inspiration and encouragement to successfully complete this project.

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<td></td>
</tr>
<tr>
<td>HPTLC</td>
<td>High-Performance Thin Layer Chromatography</td>
<td></td>
</tr>
<tr>
<td>UV</td>
<td>Ultra Violet</td>
<td></td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
<td></td>
</tr>
<tr>
<td>mg/dl</td>
<td>Milligrams per Deciliter</td>
<td></td>
</tr>
<tr>
<td>mmol/l</td>
<td>Mill mole Per Litter</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>Diabetic Mellitus</td>
<td></td>
</tr>
<tr>
<td>IDDM</td>
<td>Insulin Dependent Diabetic Mellitus</td>
<td></td>
</tr>
<tr>
<td>ppm</td>
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CHAPTER 1
INTRODUCTION

1.1 Traditional medicines

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. A number of medicinal plants, traditionally used for over 1000 years named *rasayana* are used in herbal preparations of Indian traditional health care systems.

In Indian systems of medicine most practitioners formulate and dispense their own recipes. The World Health Organization (WHO) has listed 21,000 plants used for medicinal purposes around the world.

Ancient Chinese and Egyptian papyrus writing describe the medicinal uses of plants. Indigenous cultures (such as African and Native Americans) used herbs in their healing rituals, even though they have other developed traditional medical systems. Researchers found that people in different parts of the world tends to use the same or similar plants for same purposes.

The role of traditional medicines as a solution for health problems is invaluable on a global level. This is more striking when we consider the fact that approximately 80% of the people living in less developed countries rely exclusively on traditional medicine for their health care needs. Traditional Indian and ayurvedic medical system as example, have been evolved during thousands of years and have left for posterity a well documented literary legacy which permits us to recognize immediately a
theoretical base whose conceptual framework even if were more or less archaic is found to be logical.

Most raw materials in traditional medicines are from vegetable origin and some have animal and mineral origin. However, the most important therapeutic resource is that of vegetable origin. They are qualitatively and quantitatively superior to the other two. In general, the plants are used as fresh, mainly as decoction.

The rich cultural reservoir of traditional Indian medicine is supported by diverse cultural sources, which have to be fully evaluated. This has to be considered as an important issue in our developmental efforts at the grass-root levels, as many of these living traditions still have the potential to contribute to the physical well being of our people.

1.2 Diabetes mellitus/DM

Diabetes mellitus is a disease, which occurs when the pancreas does not produce enough insulin, or when the body cannot effectively utilize the insulin it produces. This leads to an increased concentration of glucose in the blood (hyperglycemia).

1.2.1 Ayurveda concept of DM

A study of ancient literature indicates that diabetes was fairly well known and well conceived as an entity in ancient India. The knowledge of the system of DM, as the history reveals, existed with the Indians since prehistoric age. Its earliest reference (1000 BC in the ayurvedic literature) is found in mythological form where as it is said to have originated by eating ‘Havisha’, a special food which is used to be offered at the times of yagna organized by Dakshaprajapati. The disease was known as ‘Asrava’ during Vedic era (6000 BC) and a detailed description of it is available in ayurvedic
texts such as Caraka samhita, Susruta samhita. Astanga Hrida samhita (600 AD) is the first medical treatise which encompasses a clear definition of DM by mentioning about glycosuria.

DM is a disease in which urine of the patient is sweet like honey and quantitatively increased as well as of astringent, pale and with a rough quality. The whole body of the patient (Madhumehi) becomes sweet.

Sage Caraka has given a definition of DM as the diseases in which one passes urine as astringent, sweet and rough. Susruta samhita denotes DM by the term “Kshudrameha” and states that the urine in this condition resembles honey and acquires a sweet taste.

The basic Sanskrit term “Madumeha” which is used to describe DM, is composed of the words ‘madhu’+‘meha’. “Madhu” means sweet or sweetness and “meha” means excessive urination. Hence, the disease in which excessive sweet urination take place is called as Madumeha.

In ayurvedic samhita the diseases have been classified in four ways, as follows.

1. Aetiology (causes)
2. Body constitution
3. Predominance of Doshas
4. Prognosis

According to aetiology Susruta samhita has classified two types of pramehas which are mentioned below.

By birth or by genetic defect (Sahaja prameha)

Acquired (Apathyanimitta prameha)
It is believed in ayurveda texts that *Sahaja Pramehas* occurs due to defect in genetic substances either in mother or father. This clearly proves inheritance nature of the diseases. Such type of patients is always lean and thin with no other, aetiological factors. This type of DM can be compared with juvenile diabetes, as described in modern medicine. Acquired DM is due to over eating habits, especially food with sweetening agents, lack of physical exercises etc. Patients with this type are generally obese and can be compared with maturity onset type DM.

Based on the body constitution DM is classified according to the body type of patients such as, fat (*Sthula*) or with physical strength (*Balavan*) and thin (*Krisha*) and low physical strength (*Durbala*). This classification of diseases is important from the management point of view.

The predominance of doshas has classified disease into three groups, namely *Vataja Pittaja* and *Kaphaja*, these can be further sub classified into four types of *Vataja Pramehas*, six type of *Pittaja Pramehas* and ten types of *Kaphaja Pramehas*.

**1.2.2 Modern review**[3]

Diabetes mellitus is often simply referred to as Diabetes or DM—is a condition that a person has high blood sugar (glucose) level as a result of the body either not producing enough insulin, or because body cells do not properly respond to the insulin that is produced. Insulin is a hormone which is produced in the pancreas which enables body cells to absorb glucose, to turn into energy. If the body cells do not absorb the glucose, the glucose accumulates in the blood (hyperglycemia), leading to various potential medical complications.
There are many types of DM, the most common types are mentioned below.

**Type 1 diabetes:** results from the body's failure to produce insulin, and presently require injecting insulin to the person.

**Type 2 Diabetes:** results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency.

**Gestational Diabetes:** is when pregnant women, who have never had DM before, and have high blood glucose, level during pregnancy. It may proceed into developing type 2 DM.

Other forms of DM include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids.

All forms of DM have been treatable since insulin became medically available in 1921, but a cure is difficult. Pancreas transplants have been tried with limited success in type 1 DM; gastric bypass surgery has been successful in many with morbid obesity and type 2 DM; and gestational diabetes usually resolves after delivery. DM without proper treatments can cause many complications. Acute complications include hypoglycemia, diabetic ketoacidosis, or nonketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, chronic renal failure, and retinal damage. Adequate treatment of diabetes mellitus is thus important, as well as blood pressure control and lifestyle factors such as smoking cessation and maintaining a healthy body weight.
As of 2000 at least 171 million people worldwide suffer from DM, or 2.8% of the population. Type 2 DM is by far the most common, affecting 90 to 95% of the U.S. DM population.

1.2.3 Signs and symptoms

The classical symptoms of DM are polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Symptoms may develop quite rapidly (weeks or months) in type 1 DM, particularly in children. However, in type 2 DM symptoms usually develop much more slowly and may be subtle or completely absent. Type 1 DM may also cause a rapid yet significant weight loss (despite normal or even increased eating) and irreducible mental fatigue. All of these symptoms except weight loss can also manifest in type 2 DM in patients whose diabetes mellitus is poorly controlled, although unexplained weight loss may be experienced at the onset of the disease. Final diagnosis is made by measuring the blood glucose concentration.

When the glucose concentration in the blood is raised beyond its renal threshold (about 10 mmol/L, although this may be altered in certain conditions, such as pregnancy), reabsorption of glucose in the proximal renal tubuli is incomplete, and part of the glucose remains in the urine (glycosuria). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst.

Prolonged high blood glucose causes glucose absorption, which leads to changes in the shape of the lenses of the eyes, resulting in vision changes; sustained sensible glucose control usually returns the lens to its original shape. Blurred vision is a
common complaint leading to a DM diagnosis; type 1 DM should always be suspected in cases of rapid vision change, whereas with type 2 DM changes is generally more gradual, but should still be suspected.

Patients (usually with type 1 DM) may also initially present with diabetic ketoacidosis, an extreme state of metabolic dysregulation characterized by the smell of acetone on the patient's breath; a rapid, deep breathing known as Kussmaul breathing; polyuria; nausea; vomiting and abdominal pain; and any of many altered states of consciousness or arousal (such as hostility and mania or, equally, confusion and lethargy). In severe diabetic ketoacidosis, coma may follow, progressing to death. Diabetic ketoacidosis is a medical emergency and requires immediate hospitalization.

1.2.4 Causes of DM

Some of the causes of DM are mentioned below.

Lifestyle

A number of lifestyle factors are known to be important to the development of type 2 DM. In one study, those who had high levels of physical activity, a healthy diet, do not smoke, and consumed alcohol in moderation had an 82% lower rate of DM. When a normal weight was included the rate was 89% lower. In this study a healthy diet was defined as one high in fiber, with a high polyunsaturated to saturated fat ratio, and a lower mean glycemic index. Obesity has been found to contribute to approximately 55% type 2 DM, and decreasing consumption of saturated fats and trans fatty acids while replacing them with unsaturated fats may decrease the risk. The increase rate of childhood obesity in between the 1960s and 2000s is believed to have lead to the increase in type 2 DM in children and adolescents.
Environmental toxins may contribute to recent increases in the rate of type 2 DM. A positive correlation has been found between the concentrations in the urine of biphenyl A, a constituent of some plastics, and the incidence of type 2 DM.

**Genetics**

Both type 1 and type 2 DM are partly inherited. Type 1 DM may be triggered by certain infections, with some evidence pointing at Coxsackie B4 virus. There is a genetic element in individual susceptibility to some of these triggers which has been traced to particular HLA genotypes (i.e., the genetic "self" identifiers relied upon by the immune system). However, even in those who have inherited the susceptibility, type 1 DM seems to require an environmental trigger.

Various hereditary conditions may feature DM, for example myotonic dystrophy and Friedreich's ataxia. Wolfram's syndrome is an autosomal recessive neurodegenerative disorder that first becomes evident in childhood. It consists of diabetes insipidus, diabetes mellitus, optic atrophy, and deafness. Gene expression promoted by a diet of fat and glucose as well as high levels of inflammation related cytokines found in the obese results in cells that "produce fewer and smaller mitochondria than is normal," thus prone to insulin resistance.

1.3 **Ayurvedic medicines for DM**

DM was known to ancient Indian physicians as 'Madhumeha'. So many herbal products including several metals and minerals have been described for the care of DM in ancient literature. Ayurveda has been the first to give an elaborate description of this disease, its clinical features and the patterns, and its management by herbal or herb mineral drugs. It is seen that certain resistant cases of diabetes who do not respond well to modern medicines like Chlorpropamide, Tolbutamide and Glibenclamide respond
very well when treated with herbal preparations, alone or in combination with other oral hypoglycemic agents. Herbs have been shown to have hypoglycemic action in animals and humans.

The drug that was used in this research, Haridramalakee sheetha kashaya or Haridramalakee cold infusion, contains *Curcuma domestica* and *Emblica officinalis*, which are two main plant materials used for ayurvedic preparations in the treatment of DM.

### 1.3.1 Haridramalakee cold infusion

This is a type of a decoction made without the use of heat. It is used mainly for acute DM. Ayurvedic texts describe to use this medicine in the morning on an empty stomach for 6 days as a first line treatment. It is easy to administer and can be made easily. Information regarding this drug is mentioned in both Susruta samhita [5] and Astanga hrda samhita. [6]

This drug is used extensively in ayurvedic hospitals for Acute DM patients because the recovery rate is high and quick.

According to ayurvedic texts, this drug is prepared by mixing one teaspoonful of dried powder of *Curcuma domestica* with 120 ml of juice of *Emblica officinalis* and kept for one whole night (12 hours). The mixture is then filtered & filtrate is used as the medicine. [7]

In relation to ayurvedic drug preparations or *Bhaisajya Kalpana* this medicine comes under the classification of *Hima Kalpana*, which is a sub topic of “Kashaya kalpana”.

The main aim of *Kashaya Kalpana* is that to extract the active principles in to liquid by heating or without heating. Ancient scholars were capable of modifying the
raw materials such as leaves, roots, barks, etc in to the form of Swarasa (juice), Kalka (paste), Kwatha (decoction), Sheeta (cold infusion) and Phanta (hot infusion) which are commonly named as Panchavidha Kashaya Kalpana. The drug having volatile components may lose their active principles by heating. So the extraction of such type of drugs can be collected in cold infusion form or Sheetha Kashaya.\[8\]

1.4 *Emblica officinalis* Geartn. (Family EUPHORBIACEAE)

Some synonyms and usage part of *Emblica officinalis* are stated below.\[9\]

**Synonyms**

<table>
<thead>
<tr>
<th>Language</th>
<th>Synonym</th>
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<tbody>
<tr>
<td>Sinhala</td>
<td>Nelli</td>
</tr>
<tr>
<td>English</td>
<td>Amla, Emblic myrogalan, Indian Goose berry</td>
</tr>
<tr>
<td>Sanskrit</td>
<td>Aamalaki, Dhatripala, Amla, Amalaki</td>
</tr>
<tr>
<td>Hindi</td>
<td>Amla</td>
</tr>
<tr>
<td>Tamil</td>
<td>Nelli</td>
</tr>
<tr>
<td>Italian</td>
<td>Mirabolano emblica</td>
</tr>
<tr>
<td>French</td>
<td>Phyllanthe emblica</td>
</tr>
<tr>
<td>Malaysian</td>
<td>Popok Melake</td>
</tr>
</tbody>
</table>

Parts used are dried fruit, fresh fruit, seeds, leaves, root bark, and flowers.

1.4.1 **Morphological description of plant**\[9\]

A small or middle – sized tree, about 10 cm high, with a crooked trunk and spreading branches, bark thin, grey with numerous bosses whence arise the leaf-bearing branches, young parts pubescent, leaves simple, alternate, very numerous, closely placed, distiches, over lapping, spreading, nearly sessile, about 1.2 cm long, liner strap-shaped, rounded at base, sub acute, glabrous, paler beneath, stipules minute, acute, flowers unisexual, small, greenish yellow, monoecious, apetalous and axillary,
male flowers very small, numerous on slender pedicels in axillary fascicles, sepals 6, oblong, obtuse, distinct, imbricate, disc absent, stamens 3, connate throughout, female flowers few, nearly sessile, sepals as in male, ovary superior, 3-locular with 2 ovules in each loculus, surrounded by a cuplike lacerate disc, styles 3, large, recurved or spreading, lobed, fruit globose, 1.2-1.6 cm diameter, fleshy, pale green or yellow, of 3 sub-dehiscent, 2-seeded, crustaceous cocci enclosed in a thick fleshy coat, seeds 6, trigonous Diagram of *Emblica officinalis* fruit is shown in fig 1.1

**Figure 1.1 Emblica officinalis fruit**

### 1.4.2 Distribution of *Emblica officinalis*[^9]

Grows in tropical and subtropical parts of India, Ceylon, Malay peninsula and China. In Ceylon, it is very common in exposed places on and in the moist regions up to 4000 feet altitude.

### 1.4.3 Ayurvedic properties[^10]

Some of the ayurvedic properties are mentioned below.

<table>
<thead>
<tr>
<th>Rasa</th>
<th>amla, madura, kashaya, tikta, katu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guna</td>
<td>guru, ruksha, sheeta</td>
</tr>
</tbody>
</table>
Veerya - sheeta
Vipaka - madura

*Emblica officinalis* is used for diseases due to vitiated pitta dosha, headache, urine retention, disorders of the eye, gastric irritation, skin disorders, and kapha diseases. It also promotes hair growth and blood cell formation, rejuvenates the body. Increases body strength as well.

1.4.4 Pharmacognosy of fresh fruit

Fruit globosely, 2.5-3.5 cm in diameter, fleshy, smooth with six prominent lines, greenish. When tender, changing to light yellowish or pinkish color when mature, with a few dark specks. Taste sour and astringent followed by delicately sweet taste. Transverse section of mature fruit shows an epicure consisting of single layer of epidermis and 2-4 layers of hypodermis. Epidermal cells tubular in shape covered externally with a thick cuticle and appear in surface view as polygonal. Mesocarp forms bulk of fruit, this consisting of thin walled parenchymatous cells with intercellular spaces, peripheral 6-9 layers smaller, ovoid or tangentially elongated.

Rest of the cells are large in size, is diametric and radially elongated. Several collateral fibro vascular bundles are scattered throughout mesocarp consisting of xylem and phloem. Xylem is composed of tracheal elements, fiber tracheas and xylem fibers.

1.4.5 Formulations and preparations

Some of the formulation and preparations made by using *Emblica officinalis* are mentioned below.

Rasayana drugs – Brihachchhaladya ghrita, Brahmarasayana, Dhatrilauha, Dhatrirasayana,

Avaleha drugs – Chyavanaprasha, Amalakyavaleha
Arishta drugs – Phalarishta, Dhatryarishta
Kwatha drugs – Amalakyadi kwatha
Powder drugs – Triphala churna

1.4.6 Chemical constituents

The edible fruit tissue of the *Emblica officinalis* contains about three times as much protein and 160 times as much ascorbic acid as apples (*Malus pumila* Mill.). The fruit also contains considerably higher concentrations of most minerals and amino acids than apples. Glutamic acid, proline, aspartic acid, alanine and lysine constituted 29.6, 14.6, 8.1, 5.4 and 5.3%, respectively, of the total amino acids. The concentration of each amino acid, except cystine, was much higher than in apples. The *Emblica officinalis* is therefore highly nutritious, and could be an important dietary source of vitamin C, minerals and amino acids.

1.5 *Curcuma domestica* Valet. (Family ZINGIBERACEAE)

Some synonyms of *Curcuma domestica* and its used parts are given below.

**Synonyms**

<table>
<thead>
<tr>
<th>English</th>
<th>Sanskrit</th>
<th>Hindi</th>
<th>Tamil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common turmeric, Long turmeric.</td>
<td>Haridra.</td>
<td>Hildi, Halada</td>
<td>Manjal</td>
</tr>
</tbody>
</table>

Used part is Rhizomes.
1.5.1 Morphological description of plant

A biennial herb, 1-1.5 m tall with a tuberous rootstock proved with tuberiferous root fibres, rootstock and rhizomes orange-yellow, annular, cylindrical, rhizomes distichous, placed fan-wise on rootstock, 3-4 on each side, 5-7 cm long, obfuciform each terminating in a bud with several lateral branches, leaves large, lamina 28-60 cm long, 9-15 cm broad oblong, acuminate, aristate, tapering to the petiole, glabrous on both sides, bright green on the upper surface, paler below, undulate, petioles 11-22 cm long, channeled on the upper surface continued into sheaths which are 12-29 cm long and equitant, flowers bilaterally symmetrical, bisexual, yellowish-green, 5 cm long, 1.5 cm broad, 2 or 3 at various stages of growth at the axil of each bract together forming a strobilus-like pedunculate spike at the top of the rootstock, peduncle 10 cm long, spike 10-14 cm long, 3-4 lowest bracts sterile and sheathing the lower end of the strobilus, spical bracts also sterile, white, oblong to obovate, 5 cm long, 2.5 cm broad, light green to white, obtuse and recurved at the middle, all bracts fused to axis at the base and lower half of the margin, bracts of individual flowers white, linear to oblong, 2.9-3.5 cm long, 1.2-1.7 cm broad, flowers sessile, calyx-tube membranous, 1.2 cm long, segments 3, inconspicuous.

Corolla-tube funnel-shaped, limbs 3, oblong, 1.5 cm long, 0.9-1.0 cm broad, dorsal limb clawed, petaloid staminodes 3 fused to corolla-tube together forming a tube 3.7 cm long, segments free, 2-lipped, lower lip recurved and yellow in colour, upper lip of 2 segments carrying the fertile stamen with membranous filament in between, anther not crested, forming a pocked for the stigma, bases of cells spurred and incurved, stamens 3, two at the base sterile, 4 mm long, filament inconspicuous and the fertile one adnate to the staminode-tube and carried up, ovary inferior, hairy, 3-
carpellar, 3-locular with many axile ovules in each loculus, style 4.3 cm long, stigma 2-lipped, fruit not seen. Diagram of *Curcuma domestica* plant, dry and fresh rhizome is shown in fig 1.2.

![Curcuma domestica plant, dry and fresh rhizome](image)

**Figure 1.2 - Curcuma domestica plant, dry and fresh rhizome**

### 1.5.2 Distribution

A native of Southern Asia and now largely cultivated in India, Ceylon, China, Java and other tropical countries. It is grown in the mid and moist low-country in Ceylon.

### 1.5.3 Ayurvedic properties

Some of the Ayurvedic properties of *Curcuma domestica* are mentioned below.

<table>
<thead>
<tr>
<th>Rasa</th>
<th>-</th>
<th>tikta, katu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guna</td>
<td>-</td>
<td>ruksha, laghu</td>
</tr>
<tr>
<td>Veerya</td>
<td>-</td>
<td>ushna</td>
</tr>
<tr>
<td>Vipaka</td>
<td>-</td>
<td>katu</td>
</tr>
<tr>
<td>Doshagnata</td>
<td>-</td>
<td>Alleviates Vata, Pitta and Kapha</td>
</tr>
</tbody>
</table>
1.5.4 Pharmacognosy

Rhizomes are ovate, oblong, pyriform or cylindrical, often short branched, externally yellowish to yellowish-brown with root scars and annulations of leaf bases, fractured surface orange to reddish brown, odour and taste characteristic. Transverse section of rhizome shows epidermis with thick-walled cubical cells of various dimensions, a few layers of cork developed under epidermis and scattered oleo-resin cells with brownish contents, cork generally composed of 4-6 layers of thin-walled, brick shaped parenchyma, cortex characterized by the presence of mostly thin walled, rounded parenchyma cells, scattered collateral vascular bundles, vessels mainly spirally thickened. A few reticulate and annular, cells of ground tissue and cortex contain starch grains of 415µm in diameter.

1.5.5 Formulations and preparations

Some formulation and preparations of *Curcuma domestica* are mentioned below.

Oils
- Dashamoola taila, Vyaghri taila

Avaleha drugs
- Kalyanawaleha

Kanda drugs
- Haridra kanda

Kwatha drugs
- Pathyadi kwatha

Powder drugs
- Panchanimba churna

Lepa drugs
- Vridhihara lepa
1.5.6 **Chemical constituents**\(^{[14]}\)

On-line high-performance liquid chromatography–UV diode-array and electrospray mass spectrometry have been used simultaneously to analyze curcuminoids and sesquiterpenoids in a fresh turmeric (*Curcuma longa*) extract. Five major components: curcumin (1), demethoxycurcumin (2), bisdemethoxycurcumin (3), ar-turmerone (5) and curlone (6) have been unambiguously identified, based on their UV spectra, mass spectra and retention times in comparison with the data of standard compounds.

1.6 **Curcumin**\(^{[15]}\)

The three principal coloring components of curcumin that are present in various proportions are all dicinnamoylmethane derivatives. They are mentioned below.

- **1,7-Bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione**
  
  = diferuloylmethane

  Chemical formula: \(\text{C}_{21}\text{H}_{20}\text{O}_{6}\)

- **1-(4-Hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione**
  
  = p-hydroxycinnamoylferuloylmethane

  Chemical formula: \(\text{C}_{20}\text{H}_{18}\text{O}_{5}\)

- **1,7-Bis-(4-hydroxyphenyl)-hepta-1,6-diene-3,5-dione**
  
  = p, p-dihydroxydicinnamoylmethane

  Chemical formula: \(\text{C}_{19}\text{H}_{16}\text{O}_{4}\)

Structures of the three principle colouring components of Curcumin are mentioned in fig 1.3.
• Diferuloylmethane (C_{21}H_{20}O_{6}) \rightarrow R_1 = R_2 = OCH_3

• p-hydroxycinnamoylferuloylmethane (C_{20}H_{18}O_{9}) \rightarrow R_1 = OCH_3, R_2 = H

• p, p-dihidroxydicinnamoylmethane (C_{19}H_{16}O_{4}) \rightarrow R_1 = R_2 = H

![Figure 1.3 structure of three principle colouring components of curcumin](image)

Besides these major constituents, three minor constituents can be isolated which are presumed to be the geometrical isomers of compounds 1-3, above. One of these is assumed to be the cis-trans geometrical isomer of compound 1 (which has the trans-trans configuration) based on its UV spectrum, lower melting point and lower stability in solutions and in the presence of light when compared to compound 1.

1.6.1 Physico-chemical properties of curcumin

Curcumin is an oil-soluble pigment, practically insoluble in water at acidic and neutral pH, and soluble in alkali. Preparations of water-soluble curcumin by incorporation into various surfactant micellar systems (e.g. sodium dodecyl sulfate, cetylpyridinium bromide, gelatine, polysaccharides, polyethylene glycol, cyclodextrins) have been reported. In solutions the principal colouring components of curcumin exhibit keto-enol tautomerism and, depending on the solvent, up to 95 percent are in the enol form.
1.7 Other preparation for Diabetic mellitus which include *Emblica officinalis* and *Curcuma domestica* \(^{[16]}\)

Some of the preparation for diabetic mellitus which include *Emblica officinalis* and *Curcuma domestica* are mentioned below. Method of preparation of decoction – the raw material is weighed in equal quantities where the total weight is 60 grams. 960 ml of water is added and boiled until the quantity of the water becomes 240 ml.

**Decoction 1**
- Turmeric (rhizome)
- Amla (fruit)
- Aralu (fruit covering)
- Cumin (seeds)
- Malita (flowers )
- Cardamom (seeds)

**Decoction 2**
- Amla (fruit)
- Margosa (bark)
- Aralu (fruit covering)
- Vetiver (root)

**Decoction 3**
- Amla (fruit)
- Margosa (bark)
- Kumbuk (bark)
- Kelinda (fruit)
Decoction 4
Amla (fruit) - *Emblica officinalis*
Margosa (leaves) - *Azadirachta indica*
Dummella (leaves) - *Trichosanthes cucumerina*
Rasakinda (stem) - *Tinospora cordifolia*
Kalanduru (root) - *Cyperus rotundus*

Decoction 5
Amla (fruit) - *Emblica officinalis*
Mucunuwanna (leaves) - *Alternanthera sessilis*
Ingini (seeds) - *Strychnos potatorum*
Kela (stem bark) - *Bridelia retusa*
Aralu (fruit covering) - *Terminalia chebula*

Decoction 6
Turmeric (rhizome) - *Curcuma domestica*
Amla (fruit) - *Emblica officinalis*
Ingini (seeds) - *Strychnos potatorum*
Dummalla (leaves) - *Trichosanthes cucumerina*
Venivel (stem) - *Coscinium fenestratum*
Wel madata (vine) - *Rubia cordifolia*
Mango (ndosperm of fruit) - *Mangifera indica*
Diyamitta (stem) - *Cissampelos pareira*
Aralu (fruit covering) - *Terminalia chebula*
Nelum (tuber) - *Nelumbium nucifera*
Decoction 7

Turmeric (rhizome) - *Curcuma domestica*
Kekatiya (tuber) - *Aponogeton crispus*
Madan (stem bark) - *Syzygium cumini*
Babila (root) - *Sida cordifolia*
Venivel (stem) - *Coscinium fenestratum*

Decoction 8

Amla (fruit) - *Emblica officinalis*
Kaladuru (root) - *Cyperus rotundus*
Rasakinda (stem) - *Tinospora cordifolia*
Dummalla (leaves) - *Trichosanthes cucumerina*
Margosa (stem bark) - *Azadirachta indica*

Decoction 9

Turmeric (rhizome) - *Curcuma domestica*
Amla (fruit) - *Emblica officinalis*
Mucunuwanna (leaves) - *Alternanthera sessilis*
Asparagus (tuberous roots) - *Asparagus racemosa*
Licorice (stem) - *Glycyrrhiza glabra*
Sandal wood (pith) - *Pterocarpus santalinus*
Kekatiya (tuber) - *Aponogeton crispus*
Vetiver (roots) - *Vetiveria zizanioides*

Decoction 10

Turmeric (rhizome) - *Curcuma domestica*
Amla (fruit) - *Emblica officinalis*
Gokatu (whole plant) - *Hygrophila schulli*
Pethan (leaves) - *Bauhinia tomentosa*
Athividayan (stem) - *Aconitum heterophyllum*
Arjuna (stem bark) - *Terminalia arjuna*
Margosa (stem bark) - *Azadirachta indica*
Kalanduru (root) - *Cyperus rotundus*

1.8 Polyphenols[^17]

Plants produce phenolic and polyphenolic compounds as secondary metabolites. They are essential to the physiology of plants, being involved in diverse functions such as lignifications and structure pigmentation, pollination, and growth. It is considered to play a pivotal role in the pathogenesis of aging and degenerative diseases.

Oxidative stress results in oxidative alteration of biological macromolecules such as lipids, proteins and nucleic acids. In order to cope with an excess of free radicals produced upon oxidative stress, human bodies have developed sophisticated mechanisms for maintaining ‘redox-homeostasis’. These productive mechanisms include scavenging, detoxification of reactive oxygen species (ROS), blocking ROS production, sequestration of transition metals, as well as enzymatic and non-enzymatic antioxidant defenses produced in the body, that is endogenous, and other supplied with the diet, namely, exogenous ones. Among them, dietary polyphenols have been widely studied for their strong antioxidant capacities and other properties by which cell functions are regulated.
1.8.1 Classification of polyphenols

Polyphenols are the most abundant antioxidants in human diets with over 8,000 structural variants; they are secondary metabolites of plants and denote many substances with aromatic rings bearing one or more hydroxyl moieties. They are subdivided into groups by the number of polyphenolic rings and of the structural elements that link these rings. Some are mentioned below.

**Phenolic acids** - The phenolic acids with the sub classes derived from hydroxyl-benzoic acids such as Gallic acid and from hydroxyl-cinnamic acid containing caffeic, ferulic and coumaric acid.

**Flavonoids** - The largest and best studied polyphenols are the flavonoids, which include several thousand compounds, mainly divided into Anthocyanidin, present in colourful flowers and fruits and Anthoxanthins, a group of colorless compounds. Further divided categories include flavones, flavans, flavonols, flavanols, isoflavones and their glycosides. Flavonols are mainly represented by myricetin, fisetin, quercetin and kaempferol.

**Stilbenes** - Stilbenes are structurally characterized by the presence of a 1, 2-diphenylethylene nucleus with hydroxyls substituted on the aromatic rings, and exists in the form of monomers or oligomers. The best known compound is trans-resveratrol, possessing a trihydroxystilbene skeleton.

**Tannins** - Tannins are a group of water soluble polyphenols having molecular weights from 500 to 3,000 which are subdivided into condensed and hydrolysable tannins, and commonly found complexed with alkaloids, polysaccharides and proteins. On the basis of structural characteristics there are two groups, gallotannins and ellagitannins of hydrolysable tannins. The most abundant polyphenols are the condensed tannins, found
in virtually all families of plants, and comprising up to 50% of the dry weight of leaves. The convergent evolution of tannin-rich plant communities has occurred on nutrient-poor acidic soils throughout the world.

**Diferuloylmethanes** - Diferuloylmethanes are a small group of phenolic compounds with two aromatic rings substituted with hydroxyls and linked by aliphatic chain containing carbonyl groups.

**1.8.2 Anti-oxidant and free radical scavenging properties**

In order to combat and neutralize the deleterious effects of ROS, various antioxidants' strategies have evolved either by increasing the endogenous antioxidant enzyme defenses or by enhancing the non-enzymatic defenses.

Examples for antioxidant and free radical scavenging dietary polyphenols are epigallocatechin, epigallocatechingallat, epicatechingallat, hydroxytyrosol, curcumin, ellagic acid, gallic acid, corilagin, quercetin, and resveratrol.

**1.8.3 Bioactivities of dietary polyphenols**

Oxidative stress is considered to play a pivotal role in the pathogenesis of aging and several degenerative disease, type II diabetes and cancer. In order to cope with an excess of free radicals produced upon oxidative stress, humans have developed endogenous and exogenous mechanisms in order to maintain redox homeostasis. Among these, dietary polyphenols have been largely studied for their strong antioxidant capacities and other properties by which cell activities are regulated. Bioactivities of dilatory polyphenols are shown in fig1.4.
Figure 1.4 Bioactivities of dietary polyphenols
CHAPTER 2
MATERIALS AND METHODS

2.1 Instruments and Chemicals

- UV Visible spectrophotometer
  
  Model - Helios Alpha & Beta
  
  Light source - Tungsten and Deuterium
  
  Wave length - 190-1100 nm

- Distilled water

- Orthophosphoric acid, Ethanol was of laboratory grade

- Standard gallic acid solution

  In a 100 ml volumetric flask, 0.500 g of dry gallic acid was dissolved in a volume of 10 ml Ethanol and made up to 100 ml with distilled water. The solution was stored at 4°C.

- Folin-denis reagent

  Sodium tungstate - 50 g
  
  Phosphomolybdic acid - 10 g
  
  Orthophosphoric acid - 25 ml
  
  De-ionized water - 375 ml

  The above chemicals were added into a 1 liter flask fitted with a reflux condenser. The flask with several glass beads inside was refluxed on a hot plate for 2 hours, cooled and emptied into a 500 ml volumetric flask which was brought to volume with de-ionized water.
- Sodium carbonate solution

200 g of anhydrous sodium carbonate was dissolved in 800 ml of water and was boiled. After the solution was brought down to room temperature a few sodium carbonate crystals were added. This was kept for 24 hours, filtered and made up to 1 L with distilled water.

2.2 Preparation of samples

- Preparation of Gallic acid standard solution for the calibration curve

1.00 to 10.00 ml of Gallic acid stock solution was taken in 100 ml volumetric flasks and each was made up to 100 ml with distilled water.

- Preparation of Curcuma domestica (Turmeric) water extraction

Fresh samples were collected from home garden at Horana (Kaluthara District), Western province, Sri Lanka. The rhizomes were washed, cleaned, cut in to pieces of about 0.5 cm thick and air dried. The air dried rhizomes were pulverized and sieved through a soft cotton cloth. Volume of 120 ml of distilled water was mixed with 1 teaspoonful (3.045 g) of Curcuma domestica powder. Sample was hand shaken for one minute and filtered using a filter paper before analysis.

- Preparation of Emblica officinalis (Amla) pure fresh juice

Emblica officinalis were collected from a chena in Anuradhapura district. Samples were sorted out to remove damaged and contaminated fruits. Matured fresh fruits were crushed using a blender and the juice was squeezed out using a piece of cleaned and dried cotton cloth. This was vacuum filtered. 120 ml of the filtrate was used as the Emblica officinalis sample. Freshly prepared solutions were used. Before the analysis, each sample was shaken for 1 minute, filtered and 1 ml of each sample was transferred in to a 25 ml volumetric flask and made up with distilled water.
Haridramalakee cold infusion

1 teaspoonful of *Curcuma domestica* powder (3.045 g) was mixed with 120 ml of *Emblica officinalis* fresh juice. Before the analysis each sample was shaken for 1 minute, filtered and 1 ml of each sample was transferred into a 25 ml volumetric flask and made up with distilled water.

This assay was performed for 7 samples of *Curcuma domestica*, *Emblica officinalis* and Haridramalakee cold infusion for every 2 hours up to 12 hours. Before the analysis each sample was shaken for 1 minute and 1 ml of each sample was transferred into a 25 ml volumetric flask and made up with distilled water.

**2.3 Determination of total phenolic content**[18]

(Total poly-phenol assay)

Total Phenolic content was determined by Folin-Denis method. Gallic acid standard solution, blank or sample (1 ml) was mixed with 79 ml of distilled water then 5 ml of the Folin-Denis reagent was added and mixed well. This mixture was left to stand for 8 minutes at room temperature. (To allow the Folin-Denis reagent to react completely with oxidisable substances or phenolates)

15 ml of sodium carbonate solution was added to destroy the residual reagent. After incubating at room temperature for 2 hours the absorbance was measured at 755 nm using UV spectrophotometer. This assay was performed for 7 samples of *Curcuma domestica, Emblica officinalis* and Haridramalakee cold infusion every 2 hours for 12 hours. Results were expressed as parts per million (ppm).
2.4 Efficacy of Haridramalakee cold infusion in relation to mild diabetes mellitus

- DM patients who were willing to participate in the study were from the Ayurvedic Teaching Hospital at Borella.
- The following criteria were used to include or exclude the patients in the study.

2.4.1 Inclusion criteria for selection of patients

1. Patients who were willing to participate voluntarily for the study.
2. Type II Diabetes mellitus patients with Fasting Plasma Glucose levels equal or greater than 140 mg/dl of blood without any detectable/visible complications.
3. Type II diabetic patients who were newly diagnosed and have no previous medication.
4. The patients were of either sex (male or female) between the ages of 35-70 years.
5. Patients who had no other acute diseases.

2.4.2 Exclusion criteria for selection of patients

1. Pregnant or nursing patients.
2. Patients with gastro-intestinal, hepatic, cardiovascular, renal, or endocrine disorders (other than diabetes mellitus) which can interfere with the absorption, metabolism and excretion of the study herbal medicine.
3. Patients with any complication of diabetes mellitus.
4. Patient suffering from type I (IDDM) diabetes mellitus.

2.4.3 General plan of study

- The clinical Performa was given to each patient to collect data.
- The FBS samples from patients were assayed for respective glucose levels. 10 patients of type II DM with no previous medication were given medicine for 6 days. On 7th day investigated their blood samples.
• All the patients were monitored for any adverse reaction of the medicine.

• **Sample Size:** 30 patients were included in the study. But only 10 patients completed the 6 day treatment hence the final sample size remained only 10 patients.

• **Follow up:** 6 day of treatment and on the 7th day fasting blood sugar was checked.

• **Investigation:** Fasting Blood Sugar (FBS), this was done before and after treatment.
Chapter 3

Result and Discussion

3.1 Determination of polyphenolic content

Absorbance of different concentrations of gallic acid was determined at 755 nm using UV spectrophotometer. Absorbance of gallic acid at 755 nm at different concentrations is shown in table 3.1. Calibration plot for gallic acid at different concentrations is shown in figure 3.1.

Table 3.1 Absorbance at 755 nm at different concentrations of gallic acid

<table>
<thead>
<tr>
<th>Concentration of Gallic acid solution / ppm</th>
<th>Absorbance at 755 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>50.0</td>
<td>0.106</td>
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<tr>
<td>100</td>
<td>0.156</td>
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<tr>
<td>150</td>
<td>0.268</td>
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<tr>
<td>200</td>
<td>0.341</td>
</tr>
<tr>
<td>250</td>
<td>0.454</td>
</tr>
<tr>
<td>300</td>
<td>0.582</td>
</tr>
<tr>
<td>350</td>
<td>0.633</td>
</tr>
<tr>
<td>400</td>
<td>0.726</td>
</tr>
<tr>
<td>450</td>
<td>0.869</td>
</tr>
</tbody>
</table>
Figure 3.1 – Calibration plot for gallic acid
Total Phenolic content of pure *Emblica officinalis* fresh juice, *Curcuma domestica* water extraction and Haridramalakee cold infusion were calculated using the calibration curve. The values of the relevant concentrations are mentioned in table 3.2, 3.3, and 3.4 respectively.

- Pure *Emblica officinalis* fresh juice

**Table 3.2 – Concentration of total polyphenols in *Emblica officinalis* fresh juice from initial stage to 12 hours**

<table>
<thead>
<tr>
<th>Sample no:</th>
<th>hour</th>
<th>absorbance</th>
<th>Concentration / ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>initial</td>
<td>0.524</td>
<td>6895</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.549</td>
<td>7224</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.565</td>
<td>7434</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.590</td>
<td>7763</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>0.619</td>
<td>8145</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>0.651</td>
<td>8566</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>0.686</td>
<td>9026</td>
</tr>
</tbody>
</table>

- *Curcuma domestica* water extraction

**Table 3.3 – Concentration of total polyphenol in powdered *Curcuma domestica* water extraction from initial stage to 12 hours**

<table>
<thead>
<tr>
<th>Sample no:</th>
<th>hour</th>
<th>absorbance</th>
<th>Concentration ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>initial</td>
<td>0.085</td>
<td>Very low</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.070</td>
<td>Very low</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.085</td>
<td>Very low</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.089</td>
<td>Very low</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>0.093</td>
<td>Very low</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>0.086</td>
<td>Very low</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>0.083</td>
<td>Very low</td>
</tr>
</tbody>
</table>
Haridramalakee cold infusion
table 3.4 — Concentration of total polyphenol in Haridramalakee cold infusion from initial stage to 12 hours

<table>
<thead>
<tr>
<th>Sample no:</th>
<th>hour</th>
<th>Absorbance</th>
<th>Concentration ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>initial</td>
<td>0.519</td>
<td>6829</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.538</td>
<td>7079</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.554</td>
<td>7289</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.566</td>
<td>7447</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>0.575</td>
<td>7566</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>0.580</td>
<td>7632</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>0.582</td>
<td>7658</td>
</tr>
</tbody>
</table>

The medicine, Haridramalakee cold infusion was checked for polyphenol content at the initial stage and every 2 hours up to 12 hours. At the initial stage concentration was 6829 ppm, and concentration after 12 hours was 7658 ppm.

This medicine is prepared from mixing pure juice of Emblica officinalis and powdered rhizome of Curcuma domestica. Therefore the polyphenol content of both ingredients were also analyzed separately at the initial stage and every 2 hours up to 12 hours. Water extraction of powdered rhizome of Curcuma domestica was used for the analysis.

Concentration of water soluble polyphenol in Curcuma domestica water extraction was very low. From this data it is evident that most polyphenols in Curcuma domestica are not water soluble.
Concentration of *Embllica officinalis* pure juice at the initial stage was 6895 ppm and after 12 hours it was 9026 ppm. Form this it is apparent that polyphenols in this juice has increased more than the polyphenols in the Haridramalakee cold infusion. So it is evident that certain chemicals in *Curcuma domestica* powder have reduced the rate of increase in polyphenols of *Embllica officinalis* juice.

It was also revealed that the intensity of yellow colour in Haridramalakee cold infusion increased with time. Therefore it is evident that the pigment which causes the yellow colour is soluble in *Embllica officinalis* juice. This yellow pigment is a polyphenol called curcumin. So it can be assumed that curcumin dissolves in *Embllica officinalis* juice, but it did not dissolve in water since the colour change was not observable in the water extraction of *Curcuma domestica*.

The reason for polyphenols to increase with time in both Haridramalakee cold infusion and *Embllica officinalis* juice was not revealed in this study. Therefore only an assumption could be made that the polyphenols increased due to certain chemical reactions taking place in the medicine and in *Embllica officinalis* juice.
3.2 Efficacy of Haridramalakee cold infusion in relation to mild DM

- Patients selected for the study (total 10 patients):
  Males – 6
  Females – 4

- Duration of the disease:
  0-1 years – 6 patients
  2-5 years – 4 patients

- Age group of the 10 type 2 DM patients are shown in table 3.5.

Table 3.5 – age group of type 2 DM patients chosen for the study

<table>
<thead>
<tr>
<th>Age group</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>-</td>
</tr>
<tr>
<td>11-20</td>
<td>-</td>
</tr>
<tr>
<td>21-30</td>
<td>-</td>
</tr>
<tr>
<td>31-40</td>
<td>01</td>
</tr>
<tr>
<td>41-50</td>
<td>04</td>
</tr>
<tr>
<td>51-60</td>
<td>03</td>
</tr>
<tr>
<td>&gt;60</td>
<td>02</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>10</strong></td>
</tr>
</tbody>
</table>
Fasting blood glucose levels before and after treatment is shown in table 3.6.

Table 3.6 – FBS level before and after treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>FBS before treatment (mg/dl)</th>
<th>FBS after treatment (mg/dl)</th>
<th>Reduction (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>154</td>
<td>115</td>
<td>39</td>
</tr>
<tr>
<td>Patient 2</td>
<td>200</td>
<td>141</td>
<td>59</td>
</tr>
<tr>
<td>Patient 3</td>
<td>145</td>
<td>120</td>
<td>25</td>
</tr>
<tr>
<td>Patient 4</td>
<td>147</td>
<td>117</td>
<td>30</td>
</tr>
<tr>
<td>Patient 5</td>
<td>168</td>
<td>127</td>
<td>41</td>
</tr>
<tr>
<td>Patient 6</td>
<td>161</td>
<td>124</td>
<td>37</td>
</tr>
<tr>
<td>Patient 7</td>
<td>157</td>
<td>118</td>
<td>39</td>
</tr>
<tr>
<td>Patient 8</td>
<td>154</td>
<td>126</td>
<td>28</td>
</tr>
<tr>
<td>Patient 9</td>
<td>148</td>
<td>110</td>
<td>38</td>
</tr>
<tr>
<td>Patient 10</td>
<td>154</td>
<td>112</td>
<td>42</td>
</tr>
</tbody>
</table>

In regard to the data collected from the study it became evident that Haridramalakee cold infusion was effective in reducing blood glucose levels in all patients. The normal range for FBS is between 110-120 mg/dl. Patients with FBS levels below 160 mg/dl before treatment had their FBS reduced up to the normal range.

From these results it is apparent that Haridramalakee is very effective in reducing blood glucose levels and it is more effective for patients suffering from mild DM.

This medicine was tested on people having normal FBS levels in order to use a control for this study, but they complained of signs of hypoglycaemia within 2 to 3 days of consuming the medicine. So the control had to be withdrawn from the study.
CHAPTER 4
CONCLUSION

The total poly phenolic contents of the Haridramalakee cold infusion increased with time, but rate of increase is less compared to the total poly phenolic content of pure fresh *Emblica officinalis* juice. This gives an idea about the effect of turmeric powder on the rate of increase in total poly-phenols of pure fresh juice of *Emblica officinalis*.

Curcumin, a poly-phenol in turmeric powder is responsible for giving the yellow pigment. The intensity of yellow colour of Haridramalakee cold infusion increased with time suggesting that curcumin was dissolved in the juice of *Emblica officinalis*. Also the amount of curcumin dissolved increases with time. Curcumin is the known chemical component which incorporates anti-diabetic effect in this medicine. Therefore it is evident that the anti-diabetic effect of the drug increases with time.

The poly-phenols in turmeric powder dissolved very much less in water. Curcumin in the turmeric powder dissolved in juice of *Emblica officinalis*. Therefore it is evident that some water insoluble poly-phenols dissolved in the juice of *Emblica officinalis*.

Haridramalakee cold infusion was found to be effective in reducing fasting blood sugar levels mainly in patients who were suffering from mild, acute DM, who had previous fasting blood sugar levels between 140-160 mg/dl. The medicine was administered for only 6 days continuously. Therefore it is evident that the medicine has a high effect on the fasting blood sugar levels of patients with mild DM.
CHAPTER 5
SUGGESTIONS FOR FURTHER STUDIES

Quantification of curcumin in Haridramalakee cold infusion can be determined at the initial stage and after 12 hours. Also the chemical compounds in Haridramalakee cold infusion can be identified.
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